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SHORT COMMUNICATION

GC-MS AND GC-FID ANALYSIS OF VOLATILE SECONDARY METABOLITES OF THE ROOT OF *ANAPHALIS CONTORTA* HOOK F. FROM INDIA

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ABSTRACT. The chemical composition of hydro-distilled essential oil of dried roots of *Anaphalis contorta* Hook F. was analyzed for the first time by using gas chromatography equipped with flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS). The compounds of essential oil were identified according to their mass spectra and their relative retention indices determined on a capillary GC column (non-polar stationary phase). Twenty-seven compounds were identified representing 94.8% of the total oil. The major constituents were 2,2-dimethyl-2[2,4,6-trimethylphenyl] acetic acid (12.1%), labda-8,14-dien-13-ol (8.4%), δ-cadinene (8.1%), labda-7,14-dien-13-ol (6.9%), α-gurjunene (6.7%), viridiflorene (5.1%), and caryophyllene oxide (4.9%). The root essential oil of *A. contorta* produced different chemotypes.

KEY WORDS: *Anaphalis contorta* Hook F., Essential oil composition, 2,2-Dimethyl-2[2,4,6-trimethylphenyl] acetic acid. labda-8,14-dien-13-ol, GC/MS

INTRODUCTION

Essential oils are a lipophobic complex mixture of mainly terpenoids and phenylpropanoids type secondary metabolites produced by several essential oil-bearing or aromatic plants. The chemical constituents of essential oil are defined more often by the plant's organ-specific that is used for analysis or other biological activities. It is obvious that several biotic and abiotic environmental factors regulate the quality or quantity of the essential oil constituents and resulting in the biological activity affected. Individual organs or parts of many plants produced generally similar constituents as the major compound(s) or some time it was different chemical constituent(s), although the major component presents with a quantitative difference in other organs of a similar plant.

Anaphalis contorta Hook F. (Asteraceae) is widely distributed in temperate regions from Kashmir to Sikkim and Afghanistan to South West China at an altitude of 1500-4500 m. In Nainital, it is found on Cheena (Naina) peak above 2000 m [1, 2]. The fresh leaves of *A. contorta* and some other *Anaphalis* species are bruised and applied to cut wounds under a ragged bandage [3]. When taken before meals, the leaves stimulate the appetite and, as a result, it is administered to convalescents for their sedative and tonic properties [4]. The oil from the leaves of *A. contorta* showed inhibitory activity against human pathogenic bacteria and fungi [5, 6]. The oil from the leaves of *A. contorta*has been reported to possess *α*-thujene, *α*-thujone, linalyl acetate, nerol, linalool, *p*-cymene [4], 1,8 cineole, *α*-d-phellandrene, d-limonene, and fenchone [7] as the major constituents. The oil constituents from the aerial parts of *A. contorta*, caryophyllene oxide, *α*-cadinol, caryophyllene alcohol, *γ*-muurolene, 14-hydroxy-9-*epi*-β-caryophyllene, *ar*-curcumene, and β-bisabolene were the main contents [8]. However, in another study β-caryophyllene, *γ*-curcumene, *δ*-cadinene, labda-7,14-dien-13-ol, *epi*-*α*-cadinol, bulnesol, *α*-cadinol, β -bisabolol, and labda-8,14-dien-13-ol were reported as the major compounds from the essential oil of the

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flowers of *A. contorta* [9]. Besides this, the qualitative analysis of phenolic constituents of *A. contorta* has also been reported [10]. This communication presents the essential oil composition of the roots of *A. contorta* with the aim of possible utilization of the oil and demarcation of its terpenoid profile.

EXPERIMENTAL

Plant material. The roots of *A. contorta* were collected in October 2011 at an elevation of approximately 2100 m from Devidhura, district of Champawat, Uttarakhand, India. The plant was identified at the Forest Research Institute, Dehradun, where the Herbarium specimen has been deposited (Voucher No. 28/100991).

Isolation of essential oil. The dried plant material (100 g) was hydro-distilled for 3 h using a Clevenger apparatus. The oil was dried over anhydrous Na_2SO_4 and stored at -4 °C until analysis. The yield of oil was 0.03% (v/w).

Gas chromatography (GC). The GC analysis of the oil was carried out on Varian 450 gas chromatograph equipped with FID, using TG-5 column (30 m × 0.25 mm i.d., 0.25 µm film thickness) under the experimental conditions reported earlier [11, 12]. Nitrogen was a carrier gas at a 1.0 mL/min flow rate. The oven temperature was 60-220 °C at 3 °C/min, the injector and detector temperatures were 230 and 240 °C, respectively. The injection volume was 1.0 µL of 1% solution diluted in *n*-hexane; the split ratio was 1:50.

Gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis of the oil was carried out on Thermo Scientific Trace Ultra GC (Thermo Fisher Scientific Austria, Vienna, Austria) interfaced with a Thermo Scientific ITQ 1100 Mass Spectrometer (Thermo Fisher Scientific Austria) fitted with TG-5 (30m × 0.25 mm i.d., 0.25 µm film thickness) column. The oven temperature was programmed from 60 to 220°C at 3°C/min using helium as a carrier gas at 1.0 mL/min. The injector temperature was 230°C, injection volume was 0.1 µL of 1% solution prepared in *n*-hexane; the split ratio was 1:50. MS were taken at 70 eV with amass scan range of 40-450 amu. All the experimental parameters were applied from those reported earlier [13, 14].

Identification of the components. Identification of oil compounds was done based on retention index (RI, determined concerning homologous series of n-alkanes C_8-C_{25}, with similar experimental conditions), MS library search (NIST 08 MS Library (Version 2.0 f; Thermo Fisher Scientific Austria) and Wiley MS 9th Edition (Thermo Fisher Scientific Austria)), and by comparing with the MS literature data [15]. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using a correction factor.

RESULTS AND DISCUSSION

Twenty-seven compounds were characterized and identified according to their mass spectra and their relative retention indices determined on a non-polar stationary phase capillary column, comprising 94.8% of the total oil constituents. The compounds identified along with the percentage content of each constituent and its retention indices are listed in Table 1 in elution order from the TG-5 column (Figure 1).

The major constituents were identified as 2,2-dimethyl-2 [2,4,6-trimethylphenyl] acetic acid (12.1%), labda-8,14-dien-13-ol (8.4%), δ-cadinene (8.1%), labda-7,14-dien-13-ol (6.9%), α-gurjunene (6.7%), viridiflorene (5.1%) and caryophyllene oxide (4.9%). The oil was rich in sesquiterpene hydrocarbons (37.5%), followed by others (long-chain hydrocarbons, oxygenated long-chain hydrocarbons, and phenyl derivatives) (24.9%), oxygenated diterpenes (18.5%), and oxygenated sesquiterpenes (13.9%) (Table 2).

Table 1. Chemical composition of essential oil of the roots of *A. contorta.*

Compound	RT	RI	%	Identification*
Naphthalene	13.35	1151	4.9	a, b
n-Decanal	14.27	1178	t	a, b
E,*E*-2,4-Decadienal	18.85	1312	1.2	a, b
β-Maliene	21.40	1387	2.9	a, b
Isocomene	21.69	1396	1.7	a, b
n-Tridecane	22.17	1400	4.7	a, b
β-Isocomene	22.45	1418	1.2	a, b
α-Gurjunene	22.61	1423	6.7	a, b
β-Caryophyllene	23.01	1435	3.9	a, b, c
E-*β*-Farnesene	24.52	1479	1.8	a, b
epi-Cedrane	24.70	1484	1.6	a, b
γ-Gurjunene	25.12	1497	1.1	a, b
γ-Muurolene	25.30	1502	3.4	a, b
Viridiflorene	26.03	1524	5.1	a, b
δ-Cadinene	27.15	1556	8.1	a, b
Caryophyllene oxide	29.44	1624	4.9	a, b, c
β-Copaen-4-α-ol	29.52	1626	1.2	a, b
2,2-dimethyl-2[2,4,6-trimethylphenyl] acetic acid	30.57	1657	12.1	a, b
α-Cadinol	32.07	1701	4.7	a, b
1, (2,3-Dihydroxy-2-isopropenyl-2,3-dihydro-1-benzofuran-5-yl) ethanone	34.95	1786	2.0	a, b
Khusimol	35.37	1798	1.8	a, b
14-hydroxy-*δ*-Cadinene	37.59	1863	1.3	a, b
Labda-7,14-dien-13-ol	45.61	2099	6.9	a, b, d
Labda-8,14-dien-13-ol	46.30	2120	8.4	a, b, d
Nezukol	47.38	2151	3.2	a, b
cis-9-Eicosen-1-ol	50.30	2237	t	a, b
1-Eicosanol	52.09	2290	t	a, b

a = Retention index (*RI*) on TG-5 column, *b* = (GC/MS), *c* = C_o-injection of commercial samples (Sigma Aldrich chemicals Pvt.Ltd), *d* = (^{1}H- &^{13}C- NMR) [14], RT = Retention time, t = trace (<0.1%).

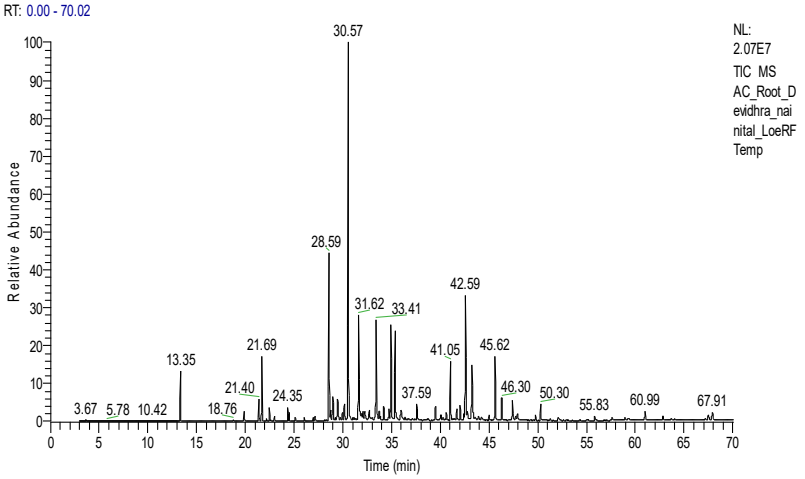

Figure 1. GC-TIC chromatogram of the essential oil of roots of *A. contorta*.

Table 2. The chemical class composition of essential oil of the roots of *A. contorta*.

Class	%
Sesquiterpene hydrocarbons	37.5
Oxygenated sesquiterpenes	13.9
Oxygenated diterpenes	18.5
Others	24.9
Total identified	94.8

It is riveting those constituents identified in the essential oil of the roots of *A. contorta* were found contrary to the aerial parts and flower oils. The compounds from the aerial parts and flower oils of this plant reported earlier were found qualitative and quantitative differences. In one report the major constituents *α*-thujene, *α*-thujone, linalyl acetate, nerol, linalool, *p*-cymene [4], while another report caryophyllene oxide, *α*-cadinol, caryophyllene alcohol, *γ*-muurolene, 14-hydroxy-9-*epi*-β-caryophyllene, *ar*-curcumene and β-bisabolene [8] from aerial parts of *A. contorta* have been reported. However, in another study β-caryophyllene, *γ*-curcumene, *δ*-cadinene, labda-7,14-dien-13-ol, *epi*-*α*-cadinol, bulnesol, *α*-cadinol, β-bisabolol and labda-8,14-dien-13-ol have been reported as the major compounds from the essential oil of the flowers of *A. contorta* [9]. It is quite interesting that according to Sinha *et al*. 1974 [7] majority of constituents were identified as monoterpenoid classes of constituents, while in another report reported no monoterpenoid constituents and described sesquiterpenoid and diterpenoid constituents [8, 9]. Besides, the roots oil in this report also represented sesquiterpenoid and diterpenoid constituents. The composition of the essential oil often changes between different plant parts [16]. The difference in the complex composition of essential oils of one kind may sometimes be hard to allocate to specific chemotypes. The formation of essential oils depends on tissue differentiation (secretory cells and excretion cavities, etc.) and the ontogenetic phase of the respective plant [17]. The secondary volatile metabolites of different parts of several plant species virtually showed quantitative differences of compounds reported in the essential oils from aerial parts and flowers of *Craniotome furcata* [18, 19], *Chromolaena odorata* [20, 21], *Crassocephalum crepidioides* [22-24], *Coleus aromaticus* [25], *Senecio belgaumensis* [26, 27], *Lantana camara* [28], *Plectranthus mollis* [29, 30] *Vernonia albicans* [31, 32] and *Vernonia cinerea* [33, 34] and sometimes derived different chemotypes from the roots of same plants [21, 23, 30, 32, 33].

CONCLUSION

In conclusion this study revealed that the roots of *A. contorta* produced different chemotypes, namely, 2,2-dimethyl-2 [2,4,6-trimethylphenyl] acetic acid, nezukol and viridiflorene, other than reported from the aerial or flowers oils. It is interesting that two diterpenoids, labda-8,14-dien-13-ol and labda-7,14-dien-13-ol are present comparatively in higher amount in the roots oil, than the aerial or flower oil of *A. contorta*. Also, the root oil of *A. contorta* presents long-chain hydrocarbons, oxygenated long-chain hydrocarbons, and phenyl derivatives, which were absent in the aerial or flower oils of *A. contorta*.

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